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6. AUTHORS
Wounjhang Park, Wei Zhang, Juliet Gopinath, Jennifer Cha and Prashant Nagpal

7. PERFORMING ORGANIZATION NAMES AND ADDRESSES
University of Colorado - Boulder
3100 Marine Street, Room 481
572 UCB
Boulder, CO 80303 -1058

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14. ABSTRACT
The PIs completed the acquisition of a nanoscale optical imaging and spectroscopy system with extremely broad spectral capability spanning the ultraviolet to the near-infrared. The equipment provides new capabilities of simultaneously obtaining high-resolution images and conducting spectroscopy on a wide variety of nanoscale or molecular materials. By providing far-field characterization of nanoscale materials, it also complements the near-field scanning optical microscope currently available in the PI’s lab.

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Report Title

Final Report: Nanoscale Optical Imaging and Spectroscopy from Visible to Mid-infrared

ABSTRACT

The PIs completed the acquisition of a nanoscale optical imaging and spectroscopy system with extremely broad spectral capability spanning the ultraviolet to the near-infrared. The equipment provides new capabilities of simultaneously obtaining high-resolution images and conducting spectroscopy on a wide variety of nanoscale or molecular materials. By providing far-field characterization of nanoscale materials, it also complements the near-field scanning optical microscope currently available in the PI's lab.

This equipment will begin making major impacts on at least three current DoD programs being conducted by the PIs: Self-Assembly of Reconfigurable By-Design Optical Materials with Molecular-Level Control (ARO W911NF-12-1-0581), Phase and Frequency Control of Laser Arrays for Pulse Synthesis (AFSOR FA9550-11-1-0026), Hierarchical Organization of Multicomponent Nanocrystals for Photodetectors through Tunable Biomolecular Interactions (ONR N000141310283).

Furthermore, an outstanding optics and materials science program exists at the University of Colorado Boulder with more than 70 faculty, many of whom have active research programs funded by the DoD and other sponsors. By providing a critical capability with wide applications, this equipment is expected to make a far-reaching and lasting impact on research on campus.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

<u>Received</u> <u>Paper</u>

TOTAL:

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

<u>Received</u> <u>Paper</u>

TOTAL:

Number of Papers published in non peer-reviewed journals:

(c) Presentations

Number of Presentations: 0.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received Paper

TOTAL:

Number of Manuscripts:

Books

Received Book

TOTAL:

Received Book Chapter

TOTAL:

Patents Submitted

Patents Awarded

Awards

Wounjhang Park, N Rex Sheppard Faculty Fellowship
Prashant Nagpal, W M Keck Foundation Research Award
Prashant Nagpal, Dean's Faculty Fellowship

Graduate Students

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
Wounjhang Park	0.00	
Jennifer Cha	0.00	
Juliet Gopinath	0.00	
Wei Zhang	0.00	
Prashant Nagpal	0.00	
FTE Equivalent:	**0.00**	
Total Number:	**5**	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: 0.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:...... 0.00

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:...... 0.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):...... 0.00

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:...... 0.00

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense 0.00

The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:...... 0.00

Names of Personnel receiving masters degrees

NAME
Total Number:

Names of personnel receiving PHDs

NAME
Total Number:

Names of other research staff

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

See attachment.

Technology Transfer

Final Report

Nanoscale optical imaging and spectroscopy from visible to mid-infrared

Prepared by:

Wounjhang Park, Jennifer Cha, Juliet Gopinath, Prashant Nagpal and Wei Zhang
University of Colorado Boulder

Prepared for:

Dr. John Prater
Army Research Office

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Aug. 15, 2014 ~ Aug. 14, 2015

Report Date:
Nov. 13, 2015

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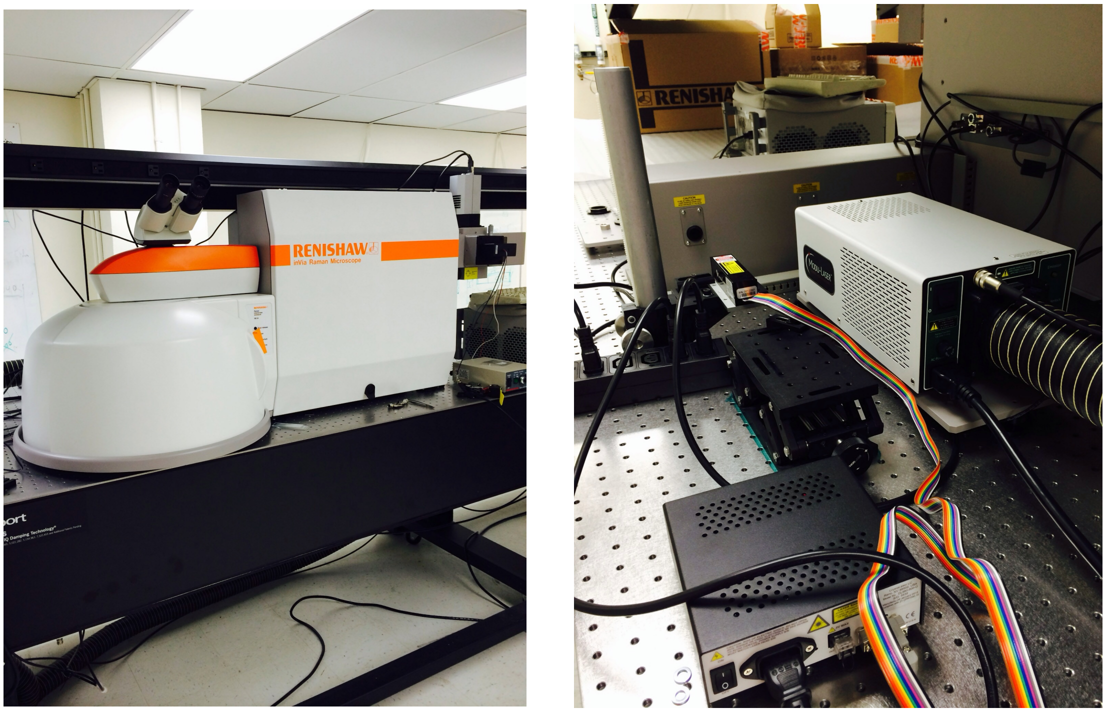
1. Acquisition of Equipment

The main piece of equipment is the Renishaw Confocal Raman Microscope system. It is comprised of a few major components: Leica DM microscope, inVia spectrometer, two CCD arrays (one for the visible and another for the near-infrared) and many other optics and electronics including the data acquisition software. For light sources we acquired two lasers, a argon ion laser with multiple lines in the visible and a 980 nm diode laser (from Crystalaser) for infrared excitation. Both lasers provide high beam quality suitable for confocal imaging. We have also acquired an additional photomultiplier tube (PMT) detector from Hamamatsu. While the CCD arrays provide spectral information in a single shot, it cannot perform time-resolved spectroscopy. PMT on the other have is a point detector and thus requires scanning to obtain full spectrum. However, it has a response time on the order of a few nanosecond and is thus suitable for time-resolved spectroscopy. Another requirement for time-resolved spectroscopy is a pulsed light source. We acquired two cw lasers because they are better suited for steady-state Raman and photoluminescence spectroscopy. We additionally acquired a fast electronic shutter from FastPulse Technology, Inc. The rise and fall time of the shutter is ~10 ns, well suited for time-resolved spectroscopy with PMT that exhibits similar response time.

Upon receiving the award, we immediately started the discussion with various vendors for performance specifications and compatibility. By February 2015, all discussions and negotiations were completed and the purchase orders were placed by March 2015. The main pieces were all delivered by May 2015. The engineers from Renishaw installed the equipment on May 27, 2015. At this time, PMT was not yet delivered and the equipment was tested for steady-state confocal Raman and photoluminescence spectroscopy only. On Aug. 25, Renishaw engineered visited again for tuning and troubleshooting and additional training for users. At this time, PMT was properly installed as well. With this, the acquisition and installation of the proposed nanoscale optical imaging and spectroscopy system was completed. The fully installed system is shown in Figure 1.

In addition, we note that this equipment was placed in a newly renovated laboratory. The University of Colorado provided ~$100K renovation fund to provide a ultralow noise environment for this equipment. The renovation was started a year earlier and included new dust-free floor, hepa filter and pressurized air handling system for clean environment, air and water systems, ultralow vibrational noise optical table with active damping control, lab benches and storages. The renovation is now complete, except for the enclosure system which will be installed on top of the optical bench to completely shield the experiments from acoustic noise. The enclosure system is expected to be delivered and installed shortly.



(a) (b)

Figure 1. Photographs of fully installed nanoscale optical imaging and spectroscopy system. (a) shows microscope with enclosure (left) and spectrometer (right). At the end of the spectrometer, three detectors (two CCD arrays and a PMT) are installed. (b) shows the two lasers: Ar ion laser and 980 nm diode laser.

2. Preliminary Results from the Newly Acquired Equipment

To demonstrate the capability of the new equipment, we conducted nanocluster photoluminescence spectroscopy on upconversion nanoparticle (UCNP) clusters. The $NaYF_4$:Yb^{3+},Er^{3+} UCNPs were synthesized using the co-precipitation method.[1-4] Briefly, a precursor total of 0.0193 mol of lanthanide chlorides (YCl_3, $YbCl_3$, and $ErCl_3$) were dissolved in 36 mL 1-octadecene and 6 mL oleic acid at 160 ^oC for 10 min then cooled to room temperature. NH_4F and NaOH were separately dissolved in methanol, added to lanthanide mixture, and vigorously stirred for 30 min. Then, the mixture was heated to 100 ^oC and degassed for 20 min. Finally, the mixture was heated up to 310 ^oC under Argon protection for 1 hr. Once the UCNP mixture was cooled to room temperature, it was washed through centrifugation with water and ethanol. We then spin coated the colloidal solution on a patterned substrate. Figure 2 shows the SEM image of a patterned substrate and two UCNP clusters, as marked by yellow circles. These nanoclusters are prepared by spin-coating the colloidal nanoparticle solutions with extremely dilute concentrations. The patterned substrate composed of a periodic array of 5 um diameter holes on silver film was fabricated by the conventional photolithography. The purpose of patterned substrate is to locate the same nanoclusters in optical microscopes as well as in the scanning electron microscope. Once the SEM images are obtained and thus the precise geometry of the nanocluster is identified, the sample is then transferred to the Raman microscope. The bright field image showed the holes and we zoom in the hole identified by SEM. We then conduct a confocal scanning of tightly focused 980 nm laser beam while taking photoluminescence spectrum at each pixel. The resulting PL map is shown in the middle panel of Figure 2. It shows two bright spots at the upper left corner and lower middle part, which coincide with the locations of the two nanoclusters of upconversion nanoparticles. Furthermore, the photoluminescence spectrum taken at each pixel clearly shows the signature of upconversion nanoparticle, exhibiting two peaks in the green and one peak in the red. These images clearly show the capability of the new microscope. This technique will be expanded to use white light source to take extinction or scattering spectrum from the gold nanoclusters being self-assembled in the two previous tasks.

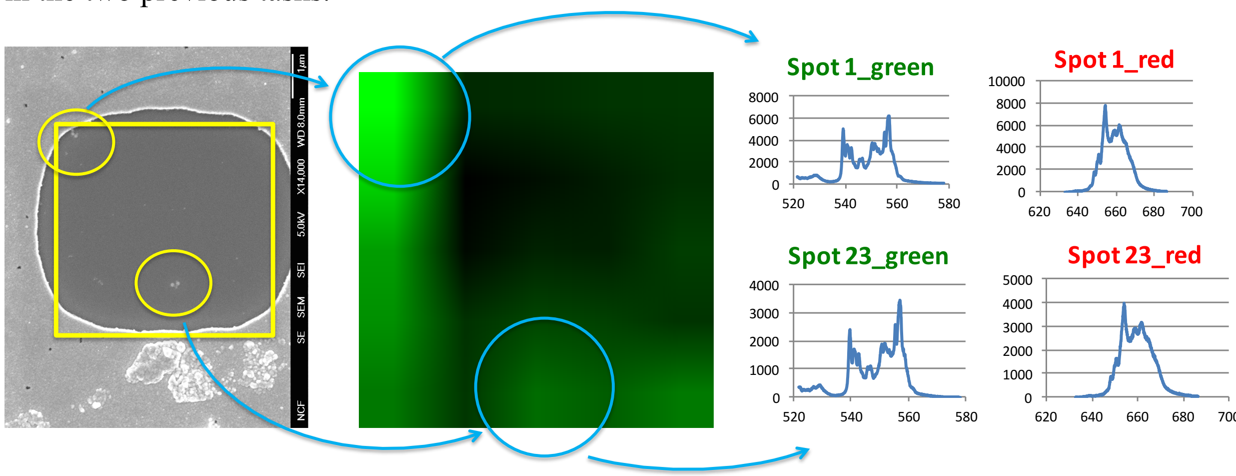


Figure 2. (Left) SEM image of a 5um size hole in a silver film and two upconversion nanoclusters in it, marked by yellow circles. (Middle) Photoluminescence map of the hole region marked by yellow square in SEM. Two bright spots show the luminescence from the two nanoclusters. (Right) Photoluminescence spectra from the nanoclusters showing the characteristic spectrum of upconversion nanoparticles.

Extending the previous work on characterizing individual nanoparticle or nanocluster, we conducted bioimaging experiments. In these experiments, the UCNPs were first treated for bioconjugation. The bioconjugation is comprised of two step processes. First the UCNP surface was modified with amphiphilic polymer, poly(maleic anhydride-*alt*-octadecene) (PMAO). For this, 80 mg of PMAO was dissolved in 1 mL of chloroform and 54 μL of methoxyethylamine (EtOMe-NH_2) was added to the mixture and stirred for 1 hr. 40mg of UCNPs in toluene were transferred to chloroform via centrifugation. The UCNPs in chloroform were then added to PMAO-EtOMe mixture and stirred for 2 hrs. Afterwards, UCNP-PMAO-EtOMe in chloroform was transferred to a 100 mL round bottom flask, vacuum dried overnight, and redispersed in water through stirring and sonication. The particles were then washed via centrifugation to remove excess polymer. After PAMO coating is completed, C-225, an antibody to epidermal growth factor receptor, was attached as follows. 0.5 mL of PMAO-EtOMe coated UCNPs were added to 0.5 mL of 0.1 M MES buffer. The solution was then combined with EDAC and incubated in room temperature. The mixture was then washed via centrifugation. 0.5 mL of PBS containing 2 mg/mL C-225 was added to above UCNP solution (roughly 10 mg/mL) and nutated overnight at 4 ^oC. The conjugated UCNPs were centrifuged at 12 kG for 5 min and re-suspended in 0.05% Tween20.

After the bioconjugation is completed, we conducted cell binding assay as follows.[5] Human bladder cancer cells HTB9 (American Type Culture Collection, Manassas, VA, USA) and primary bladder culture were grown in OptiMEM (Eibco, Grand Island, NY, USA) with 3.75% fetal bovine serum (Gemini, Woodland, CA, USA) and 100 units streptomycin-penicillin sulphate (Life Technologies, Grand Island, NY, USA) in chamber slides. Cells were incubated at 37 ^oC with 5% CO_2. On the next day, cells were incubated with C-225 antibody conjugated UCNPs for two hours, and then the medium was removed and washed three times with PBS. The cells were then fixed in 2% formalin and probed with DyLight labeled secondary antibody against human IgG (Jackson Lab). Monitoring and imaging the binding of C-225 antibody labeled with DyLight labeled secondary antibody with HTB9 bladder cancer cell was conducted with a fluorescent microscope (Nikon Eclipse TE2000-S).

Finally, the UCNP treated cells were imaged by the Raman microscope system acquired in this grant. Figure 3 shows bright field optical micrograph, upconverted fluorescence micrograph and the combined image. As shown, the UCNPs are attached to cell membrane and exhibit bright upconverted fluorescence under 980 nm infrared excitation. It should be noted that the background is complete dark and absent of any autofluorescence, which is ubiquitous in conventional fluorescence microscopy using high energy ultraviolet light. Thus the UCNP imaging produces high contrast images with cell targeting functionality provided by the bioconjugation.

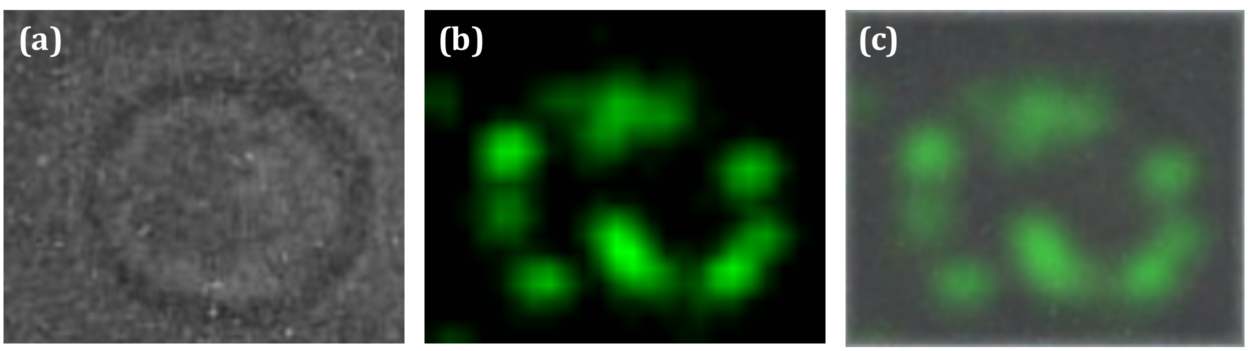


Figure 3. Microscope images of UCNP treated bladder cancer cell. (a) Bright field micrograph, (b) Upconverted fluorescence micrograph obtained by the scanning confocal microscope using 980 nm laser, and (c) Combined image of bright field and upconverted fluorescence.

3. Impact on the Research and Education Programs

Since we just completed the full installation of the equipment, we do not yet have any products to report such as academic publications, conference presentations and students graduated. However, the new capability provided by this equipment will have a long lasting impact on broad research and education programs at the University of Colorado Boulder. Many of the DoD programs described in the proposal are continuing and will benefit directly from this new capability. Many other programs funded by other government agencies including National Institute of Health and Department of Energy will also be impacted directly. The University of Colorado Boulder campus has an outstanding optics and materials science program with more than 70 faculty, all of whom have active research programs funded by the DoD and other sponsors. By providing a critical capability with wide applications, this equipment is expected to make a far-reaching and lasting impact on research on campus. Furthermore, the research activities naturally provide excellent opportunities for training graduate and undergraduate students as well as postdoctoral scholars, building high quality workforce to the industry relevant to the missions of DoD agencies and others.

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